The Neuropathology of Aminergic Nuclei in Alzheimer's Disease

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Neuronal loss and the presence of neurofibrillary tangles (NFTs) within aminergic nuclei were examined in a series of patients with Alzheimer's disease (AD). Neuromelanin-containing neurons within the locus ceruleus and large nucleolus-containing neurons within the dorsal raphe nucleus and the central superior (raphe) nucleus were counted in 25 patients with AD and in 12 age-matched control subjects. Numbers of NFTs were quantified in the same regions. Counts were compared with clinical data, including psychiatric evaluations, available for 21 of the patients with AD. Within the locus ceruleus in the patients with AD, abnormalities were more severe at mid level than at caudal or rostral levels ($p < 0.01$). Within the dorsal raphe nucleus, neuronal loss was most severe caudally ($p < 0.05$). NFTs, but not neuronal loss, were demonstrated within the central superior nucleus. Neuronal and NFT counts did not correlate at individual levels; the relative severity of both pathological processes was consistent from level to level within nuclei but was less consistent between nuclei. Neuronal loss correlated inversely with age, particularly within the locus ceruleus. Duration of disease correlated inversely with counts of NFTs, particularly within the dorsal raphe nucleus, implying a correlation between NFT counts and rate of progression of disease as all but 3 patients had severe dementia. Significantly, patients with AD complicated by major depression had fewer neurons at the mid level of the locus ceruleus and at the rostral level of the central superior nucleus in comparison with nondepressed patients. There was a trend suggesting greater loss of neurons at all levels of the locus ceruleus and dorsal raphe nucleus in depressed individuals.

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Neurons in the locus ceruleus (LC) and cortically projecting raphe nuclei are frequently affected in Alzheimer's disease (AD) [1–5]. Loss of neuromelanin-containing, noradrenergic neurons and formation of neurofibrillary tangles (NFTs) occur throughout the rostrocaudal extent of the LC [6, 7], although neuronal loss has been recently reported to be less severe within the caudal and ventral parts of this nucleus [4, 8]. NFTs also occur commonly within the dorsal raphe nucleus (DR) and the central superior (raphe) nucleus (CSN) in patients with AD, and a number of investigators have documented reductions in numbers of large, putative serotonergic neurons within these nuclei [1, 5, 9, 10]. However, only a limited number of studies have attempted to determine clinical and pathological correlates of severity of involvement of the LC, the DR, and the CSN [9, 11–16].

Psychiatric complications, including major depression, commonly occur in patients with AD [17–19]. While the normal functions of central aminergic systems are only poorly understood (e.g., see [20]), several lines of evidence have implicated aminergic systems in depression [21]. However, studies in patients with AD regarding possible relationships between the extent of involvement of these systems and the presence of depression have not been previously reported.

The objective of the present study was to evaluate comprehensively the pathological involvement of the LC, the DR, and the CSN in a series of aged control subjects and patients with AD for whom detailed clinical information, particularly psychiatric evaluations, was available. Neurons and NFTs were counted at multiple levels within each nucleus to determine the following: (1) the extent of neuronal loss and number of NFTs in these cortically projecting aminergic nuclei in AD; (2) the patterns of involvement at different

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levels of these nuclei; (3) the relationships between severity of involvement at one level of a nucleus in an individual and severity of involvement at other levels; and (4) the correlations between the severity of involvement of one nucleus in an individual with the severity of involvement of other nuclei. Finally, we attempted to relate pathological measures with specific clinical features, including age at death, duration of illness, and the presence of depression. Preliminary data from a portion of this study have been submitted for publication in a brief communication.

Materials and Methods

Case Material

Formalin-fixed brain tissue was obtained from the Brain Resource Center of the Neuropathology Laboratory at The Johns Hopkins Hospital from 25 patients diagnosed as having AD (mean age 72.5 years; range 55–88; 14 men, 11 women) and 12 aged control subjects (mean age 68.9 years, range 45–96, 4 men, 8 women). A pathological diagnosis of AD was made by histological demonstration of numerous senile plaques and NFTs in the neocortex, amygdala, and hippocampus. Fifteen plaques or more per low-power (× 100) field in the neocortex was required [22–24]. Other causes of dementia, including multiple infarcts and Parkinson's disease, were absent. Twenty-one patients had a clinical diagnosis of AD; 4 patients with dementia had been clinically diagnosed as having multiple infarct dementia or Pick's disease but proved to have AD upon pathological examination. Control subjects had no history of neurological disease, and their brains demonstrated no significant pathological changes.

Clinical Assessment

Twenty-one of the patients with AD were followed at the Dementia Research Clinic at The Johns Hopkins Hospital (Department of Psychiatry), and the extent of dementia and psychiatric complications were regularly assessed [C. A. Ross, G. Pearson, R. Zweig, and colleagues, unpublished data]. Based on chart review and structured retrospective interviews with informed family members (performed without knowledge of the pathological findings), information obtained for this study included age of onset, duration of disease, last recorded Mini-Mental State (MMS) score [25] prior to terminal illness, presence of major depression (DSM-III criteria [26]), and presence of hallucinations.

Tissue Processing and Staining

One or more blocks of brainstem from each case containing LC, DR, and/or CSN cut in the transverse plane were dehydrated, embedded in paraffin, and sectioned serially (12 μm). Every twentieth section was stained with cresyl violet; additional sections from selected levels (see text following) were stained with Congo red or Bielschowsky's silver stain.

Selection of Slides and Definition of Boundaries of Nuclear Groups

Three levels of LC, three levels of DR, and two levels of CSN were defined using, as criteria, density (in control cases)

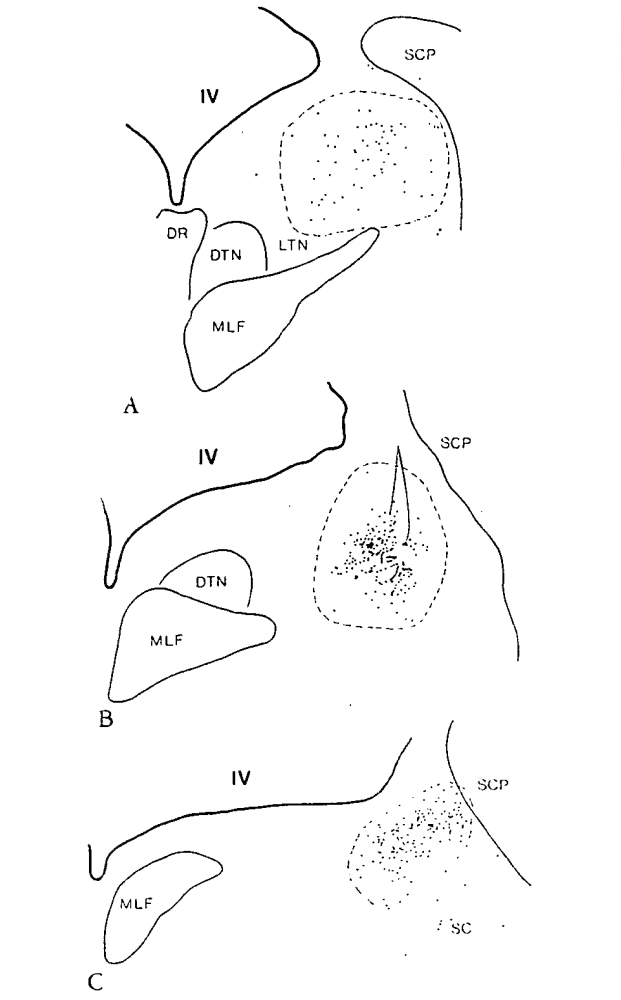

Fig 1. Camera lucida drawings demonstrating levels and boundaries of the locus ceruleus (LC) from a control subject (right side). (A) Rostral level: pigmented neurons, loosely packed in a circular distribution, are located dorsolateral to the laterodorsal tegmental nucleus (LTN), the dorsal tegmental nucleus of Gudden (DTN), and the most caudal dorsal raphe nucleus (DR). This level corresponds to Olszewski and Baxter (O&B) plate 28 [49]. (B) Mid level: pigmented neurons, densely packed in a diamond-shaped distribution, are located caudal to the DR, lateral to the caudal DTN, and dorsal to the nucleus reticularis tegmenti pontis. (C) Caudal level: pigmented neurons are more densely packed dorsally than ventrally in a rectangular distribution elongated along a ventromedial-dorsolateral axis. Corresponds to O&B plate 26. At all levels, boundaries are defined by the distribution of pigmented neurons (excluding subceruleus neurons at the caudal level). (MLF = *medial longitudinal fasciculus;* SCP = *superior cerebellar peduncle;* IV = *4th ventricle [see text]*). (×22 *before 20% reduction.*)

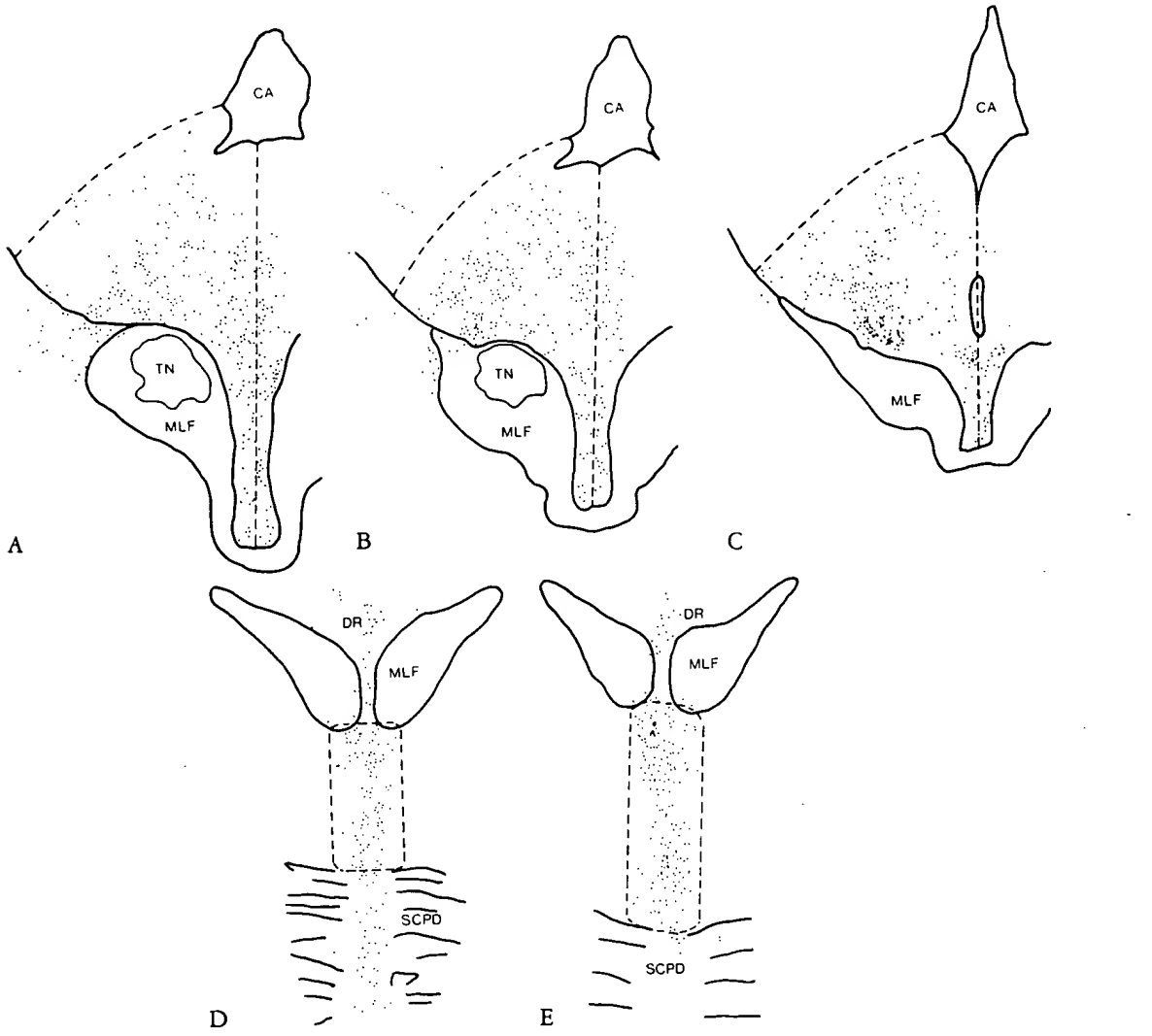

Fig 2. Camera lucida drawings demonstrating levels and boundaries of the dorsal raphe nucleus (DR) (left side) and the central superior (raphe) nucleus (CSN) from control subjects. (A) Rostral level: the DR is located caudal to the oculomotor nucleus, dorsal to the rostral trochlear nucleus (TN), and includes a prominent group of large neurons overlapping the periaqueductal gray margin ventrolaterally. Corresponds to O&B plate 32. (B) Mid level: the DR is located dorsal to mid TN and includes a prominent group of large neurons extending dorsomedially from the ventrolateral margin. (C) Caudal level: the DR is located caudal to the TN, rostral to the 4th ventricle, and includes a prominent group of large neurons ventrally. At all levels of the DR, boundaries are defined by the MLF, periaqueductal gray margin, and a curved line extending ventrolateral from the mid cerebral aqueduct (CA). (D) Rostral level: the CSN appears as densely packed neurons extending ventrally from the MLF (one-half the distance to the medial lemniscus and located dorsal to caudal SCP decussation (SCPD) (corresponds to O&B plate 38). (E) Caudal level: CSN appears as densely packed neurons that extend ventrally almost to the medial lemniscus and are located dorsal to most caudal SCPD. At all levels of the CSN, boundaries are defined by the ventral MLF and dorsal SCPD. (Abbreviations same as in Fig 1.) (×22 before 20% reduction.)

and distribution of neurons of interest at each level and relationships to neighboring structures. Specific criteria used to guide selection of levels and to determine boundary lines are illustrated in Figures 1 and 2. The number of levels available from each nucleus for any case varied (Table 1). In those cases where sections of both left and right LC and/or DR were available, all levels selected from individual nuclei were from the same side. Selection of slides and placement of boundary lines were performed without knowledge of clinical assessments of individual cases.

Counts of Neurons and NFTs

Neurons and NFTs were counted within designated areas of coded slides independently by two investigators. Counts per unit area were not determined because of the heterogeneous distribution of neurons and NFTs within the nuclei. In the LC, all neuromelanin-containing neurons (> 12.5 μm in greatest diameter) were counted (×400). The presence of a nucleolus was not required for a neuron to be counted because neuromelanin often obscures this structure. Cells smaller than 12.5 μm were excluded because of the difficulty

Table 1. Neuronal and NFT Counts at Three Levels of the LC and DR and Two Levels of CSN in Control Subjects and AD Patients

Nucleus	Level	Control Subjects		AD Patients	
		Neurons	NFTs	Neurons	NFTs
LC	Rostral	54.1 ± 21.4 (7)[a]	3.4 ± 4.2 (4)	16.4 ± 13.1 (14)**[b]	12.0 ± 7.6 (11)*
	Mid	140.2 ± 41.4 (9)	1.8 ± 1.8 (7)	26.6 ± 18.6 (20)**	17.0 ± 10.7 (19)**
	Caudal	147.4 ± 35.1 (9)	1.2 ± 1.3 (7)	49.3 ± 20.3 (15)**	10.2 ± 7.7 (14)**
DR	Rostral	105.2 ± 47.5 (6)	4.9 ± 3.9 (6)	95.5 ± 57.8 (13)	47.3 ± 32.0 (13)**
	Mid	114.5 ± 46.1 (6)	5.9 ± 5.6 (6)	86.3 ± 55.7 (16)	65.0 ± 33.3 (13)**
	Caudal	103.2 ± 34.7 (7)	12.5 ± 13.1 (6)	66.5 ± 29.9 (12)*	61.5 ± 40.6 (12)**
CSN	Rostral	51.8 ± 25.5 (7)	3.2 ± 3.0 (5)	46.7 ± 13.3 (9)	20.6 ± 6.7 (9)**
	Caudal	67.3 ± 23.1 (6)	4.1 ± 3.3 (4)	74.5 ± 25.8 (8)	28.9 ± 16.3 (8)**

[a]Data are expressed as means ± standard deviation. Numbers of cases are in parentheses.
[b]Statistical comparisons of counts: * = $p < 0.05$, ** = $p < 0.01$.

NFT = neurofibrillary tangle; LC = locus ceruleus; DR = dorsal raphe; CSN = central superior (raphe) nucleus; AD = Alzheimer's disease.

sometimes encountered in distinguishing small neurons from large extracellular neuromelanin fragments or macrophages containing these fragments, particularly in cases of disease. All nucleolus-containing neurons (> 25 μm in greatest diameter) within areas designated as DR and CSN were counted (×400).

Congophilic/birefringent NFTs (intracellular and extracellular) were counted from Congo red–stained sections at each level of the LC (×250). A silver stain was not used for counting NFTs in this nucleus because intense silver staining of neuromelanin often obscures NFTs. NFTs (intracellular and extracellular) were counted from Bielschowsky-stained sections at each level of the DR and the CSN (×400).

Statistical Methods

Comparisons between groups were made using Student's *t* test (two-tailed). Lack of counts at each level for every case precluded use of analysis of variance. Relationships between groups were also assessed using both Pearson's product-moment correlation coefficient (*r*) and Spearman's rank order correlation coefficient. Although only *r* values are reported, all correlations reported as significant were so using both correlation coefficient measures. All *t* tests and correlation analyses for results at two levels included only subjects with counts at both levels. Dividing individual neuronal counts of patients with AD by the mean neuronal count of control subjects at the same level provided a measure of neuronal survival or, conversely, neuronal loss for the purpose of level-to-level comparisons (*t* tests). In all comparisons reported as significant, $p < 0.05$ except where noted. Counts by the two investigators were consistently highly correlated (*r* ranged from 0.85 to 0.97); all statistical analyses were performed on the means of these scores.

Results

Counts of Neurons and NFTs in LC of Control Subjects and Cases of AD

Up to three levels of the LC were studied in 22 patients with AD and 12 control subjects. Neuronal loss

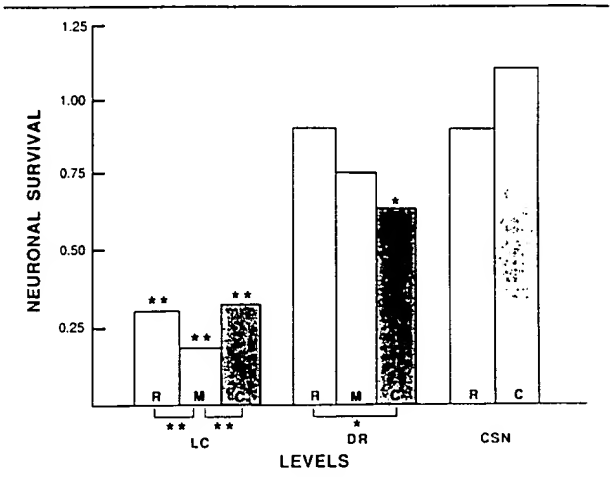

Fig 3. Bar graph of mean neuronal counts of patients with Alzheimer's disease (AD) divided by mean neuronal counts of control subjects (neuronal survival) at three levels of the locus ceruleus (LC), three levels of the dorsal raphe nucleus (DR), and two levels of the central superior (raphe) nucleus (CSN). Statistical comparisons of counts from AD and control cases are indicated above bars. Statistical comparisons of neuronal survival between levels within nuclei are indicated by lines connecting bars below the baseline (= $p < 0.05$, ** = $p < 0.01$).* (R = *rostral;* M = *mid;* C = *caudal.*)

was highly significant ($p < 0.01$) at all levels of this nucleus in patients with AD but was most severe at the mid level, with neuronal counts averaging 30%, 19%, and 33% of control values for rostral, mid, and caudal levels, respectively (see Table 1, Fig 3). Differences between neuronal survival (or, conversely, neuronal loss) at mid level in comparison to rostral and caudal levels were highly significant ($p < 0.01$). Counts of NFTs, significantly higher in patients with AD than in control subjects, were also highest at the mid level (see

Table 2. Pearson Correlations Between Neuronal Counts in AD Patients at all Levels[a]

	Locus Ceruleus			Dorsal Raphe Nucleus			Central Superior (Raphe) Nucleus
	Rostral	Mid	Caudal	Rostral	Mid	Caudal	Rostral
Locus ceruleus							
Rostral							
Mid	0.83**						
Caudal	0.63	0.63*					
Dorsal raphe nucleus							
Rostral	0.05	0.15	0.16				
Mid	0.13	0.27	0.25	0.97**			
Caudal	−0.08	0.57	0.36	0.51	0.68*		
Central superior (raphe) nucleus							
Rostral	0.91**	0.88**	0.59	0.47	0.30	0.23	
Caudal	−0.09	0.09	0.56	−0.23	−0.34	−0.60	0.17

[a]Significance (* = $p < 0.05$, ** = $p < 0.01$) is indicated only if Spearman's rank order correlation coefficient is also $p < 0.05$. Numbers of cases are not shown.

AD = Alzheimer's disease.

Table 3. Pearson Correlations Between NFT Counts in Patients at all Levels[a]

	Locus Ceruleus			Dorsal Raphe Nucleus			Central Superior (Raphe) Nucleus
	Rostral	Mid	Caudal	Rostral	Mid	Caudal	Rostral
Locus ceruleus							
Rostral							
Mid	0.83**						
Caudal	0.74	0.43					
Dorsal raphe nucleus							
Rostral	0.86	0.46	0.79				
Mid	0.90**	0.52	0.82**	0.90**			
Caudal	0.77*	0.59	0.78	0.95	0.84**		
Central superior (raphe) nucleus							
Rostral	0.48	0.27	−0.25	0.37	0.61	0.81*	
Caudal	0.67	−0.13	−0.52	0.82	0.21	0.50	−0.22

[a]Significance (* = $p < 0.05$, ** = $p < 0.01$) is indicated only if Spearman's rank order correlation coefficient is also $p < 0.05$. Numbers of cases are not shown.

NFT = neurofibrillary tangle; AD = Alzheimer's disease.

Table 1) and were significantly higher at this level than at the caudal level.

In individual AD patients, no relationship was apparent between neuronal and NFT counts at any level of the LC or other nuclei studied (data not shown). Whereas, for a given individual, the severity of pathological changes varied from level to level, individuals with high (or low) counts at one level relative to other individuals also had high (or low) counts at all other levels. Correlations between neuronal counts in patients with AD at different levels of the LC and other nuclei studied are given in Table 2. Correlations between NFT counts at different levels are given in Table 3.

Counts of Neurons and NFTs in the Raphe of Controls and Patients with AD

Up to three levels of the DR were studied in 18 patients with AD and 7 control subjects; up to two levels of the CSN were studied in 10 patients with AD and 7 control subjects. Within the DR, neuronal loss in those with AD was minimal at the rostral level, greater (but not significant) at the mid level, and most severe at the caudal level (64% of control value, $p < 0.05$). The difference between neuronal survival at rostral and caudal levels was significant. Within the CSN, neuronal counts in the patients with AD were similar to those of control subjects at both levels. Counts of NFTs were much higher in those with AD than in the

Table 4. Pearson Correlations in AD Patients of Age at Death and Duration of Disease with Numbers of Neurons and NFTs Counted at all Levels[a]

Nucleus	Level	Age and No. of Neurons	Age and No. of NFTs	Duration and No. of Neurons	Duration and No. of NFTs
Locus ceruleus	Rostral	0.57*	−0.24	0.08	−0.69
	Mid	0.55*	0.32	0.04	−0.14
	Caudal	0.10	−0.24	−0.09	−0.54
Dorsal raphe nucleus	Rostral	0.16	−0.31	0.10	−0.79**
	Mid	0.36	−0.35	0.11	−0.68*
	Caudal	0.45	−0.12	−0.04	−0.81**
Central superior (raphe) nucleus	Rostral	0.25	−0.01	−0.07	−0.43
	Caudal	−0.70	−0.16	−0.10	−0.56

[a]Significance (* = $p < 0.5$, ** = $p < 0.01$) is indicated only if Spearman's rank order correlation coefficient is also $p < 0.05$. Numbers of cases are not shown.

AD = Alzheimer's disease; NFTs = neurofibrillary tangles.

control subjects at all levels of the DR and CSN ($p < 0.005$) (see Table 1). Patients with AD with high (or low) counts at one level of the DR also had high (or low) counts at all other DR levels.

Relationships Between Parameters Measured in Aminergic Nuclei in Patients with AD

Most correlations of neuronal counts in different nuclei were positive, but this correlation was significant ($p < 0.01$) only for the comparisons of rostral LC/rostral CSN and mid LC/rostral CSN (see Table 2). Thus, our data indicate that the relative extent of neuronal loss in one nucleus in individual cases only weakly reflects the relative severity of neuronal loss in other nuclei. Similarly, numbers of NFTs in the DR correlated positively with numbers of NFTs in the LC for all nine level-to-level comparisons; three of these correlations were significant. Correlations between counts of NFTs within the CSN and DR were also positive and significant for the comparison of caudal DR/rostral CSN (see Table 3).

Relationships Between Changes in Aminergic Nuclei and Age, Duration of Disease, and MMS Scores in Patients with AD

Counts of neurons correlated positively and significantly with age at death at rostral and mid levels of the LC. This correlation was also positive, but not significant, at all other levels with the exception of the caudal CSN. NFT counts correlated negatively and significantly with duration of disease at all three levels of the DR. This correlation was negative, but not significant, at all other levels (Table 4). There were no significant correlations between neuronal counts and duration of disease, NFT counts and age at death, or age at death and duration of illness. For those correlations that were significant, points were distributed continuously (i.e., no evidence of a bimodal distribution). Most of those with AD had severe dementia (MMS scores 0–2 in 17 cases and 5 in 1 case), although 3 patients had only moderate dementia with MMS scores of 7, 10, and 16. In 2 of the moderately demented patients, DR neuronal counts were at or above control levels. Otherwise, no relationship was apparent between MMS score and counts of neurons (Figs 4, 5) or NFTs (data not shown).

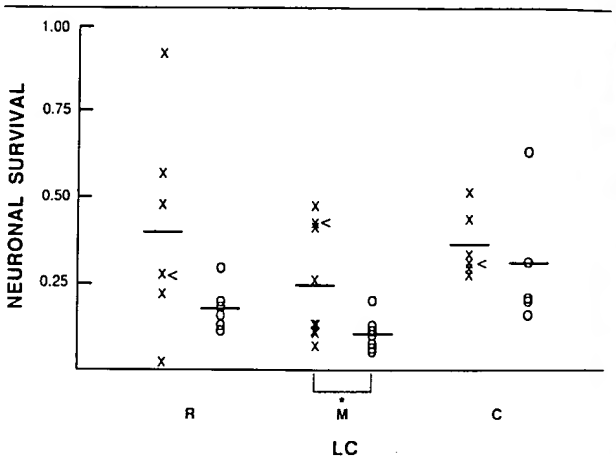

Fig 4. Scatterplot comparing neuronal counts at three levels of the locus ceruleus (LC) in individual nondepressed and depressed patients with Alzheimer's disease divided by mean neuronal counts of control subjects. X = *nondepressed;* O = *depressed;* < = *cases with moderate dementia. Bars indicate means. Statistical comparisons are indicated by lines connecting the columns below the baseline* (* = $p < 0.05$). (R = *rostral;* M = *mid;* C = *caudal.*)

Relationships with Presence of Depression and Hallucinations in Patients with AD

Of the patients with AD, those whose course was complicated by major depression (8 patients) had significantly fewer neurons at the mid LC and rostral CSN

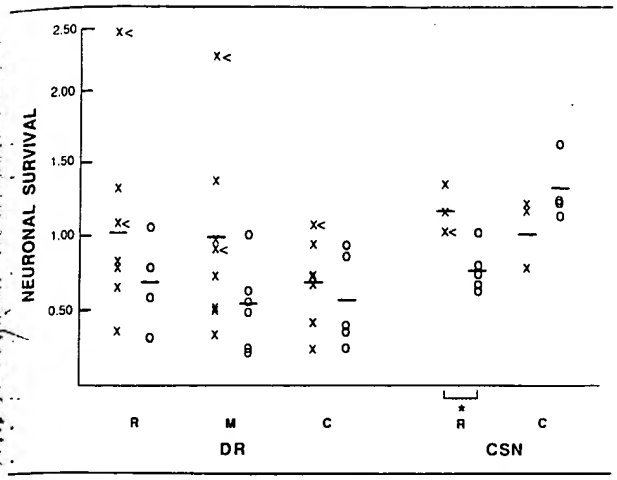

Fig 5. Scatterplot comparing neuronal counts at three levels of the dorsal raphe nucleus (DR) and two levels of the central superior (raphe) nucleus (CSN) in individual nondepressed and depressed cases of Alzheimer's disease divided by mean neuronal counts of control subjects. X = *nondepressed;* O = *depressed;* < = *cases with moderate dementia. Bars indicate means. Statistical comparisons are indicated by lines connecting the columns below the baseline* (* = $p < 0.05$). (R = *rostral;* M = *mid;* C = *caudal.*)

levels when compared with the 13 patients without this complication. With the exception of the caudal CSN, fewer neurons were also counted in the cases with depression at all other levels, but differences were not significant (see Figs 4, 5). Although neuronal counts in nondepressed patients with AD were significantly less than control counts at all three levels of the LC, there were no significant differences between neuronal counts in control and nondepressed patients with AD at any level of the DR. Significant differences were not demonstrated for neuronal counts and the presence of hallucinations or for NFT counts and the presence of either depression or hallucinations (data not shown).

Interactions Between Depression and Other Features in Patients with AD

Patients with AD complicated by depression were younger at death than those without this complication. This age difference was smallest (1.3 years) for those with the mid LC level evaluated, although small sample sizes precluded statistical analysis of the contribution of age to differences in counts of neurons between depressed and nondepressed individuals at this or at any other level. Age differences between depressed and nondepressed patients with AD were significant ($p < 0.01$) for those in whom mid and caudal levels of the DR were evaluated. Duration of illness was slightly, but not significantly, less in the depressed group (Table 5). Neither of the 2 patients with AD who had moderate dementia and neuronal counts within the DR at or above control levels were depressed (see Fig 5).

Discussion

Transmitter Specificity of Neuronal Populations Studied

In the adult human brain, the presence of neuromelanin within a neuron strongly implies that the neuron is catecholaminergic [27]. Neuromelanin is easily distinguished from lipofuscin and other neuronal pigments using the cresyl violet stain. Although there is evidence that within certain brainstem regions some catecholaminergic neurons may contain little neuromelanin [28], the correspondence between presence of neuromelanin and dopamine-beta-hydroxylase immunoreactivity (a specific marker for noradrenergic neurons) within the LC is quite good [7]. Thus, counts of neurons within the LC probably correspond closely to numbers of noradrenergic neurons.

In contrast to the LC, the transmitter specificity of neurons counted within the DR and CSN is far less certain, although in the macaque brainstem, a high proportion of the neurons located within those regions of the DR studied contain serotonin-histofluorescence [29]. Moreover, most of the medium- to large-sized

Table 5. Age at Death and Duration of Disease for Those Depressed and Nondepressed Patients Studied at Each Level[a]

		Depressed			Nondepressed		
Nucleus	Level	n	Age	Duration	n	Age	Duration
Locus ceruleus	Rostral	6	67.1 ± 3.9	6.5 ± 2.6	4	73.1 ± 6.4	8.8 ± 1.7
	Mid	7	69.0 ± 5.6	7.1 ± 2.8	9	70.3 ± 7.9	8.0 ± 2.2
	Caudal	5	70.2 ± 6.3	6.6 ± 3.2	6	74.0 ± 8.2	8.5 ± 2.4
Dorsal raphe	Rostral	4	68.7 ± 4.1	7.2 ± 2.6	7	74.1 ± 3.9	8.4 ± 1.7
	Mid	6	67.3 ± 3.9	6.7 ± 2.7	8	74.7 ± 4.0*	8.1 ± 1.8
	Caudal	5	66.6 ± 2.1	6.6 ± 2.5	7	75.0 ± 4.4*	7.4 ± 1.5
Central superior (raphe)	Rostral	5	68.4 ± 3.6	7.0 ± 1.9	3	77.0 ± 5.3	7.7 ± 1.5
	Caudal	4	68.7 ± 4.1	6.7 ± 2.1	3	73.6 ± 4.6	8.0 ± 1.7
Total		8	68.8 ± 5.3	7.3 ± 2.6	13	73.1 ± 8.2	8.2 ± 2.0

[a]Data are expressed as means ± standard deviation. n = Numbers of cases in each group. Statistical comparisons are indicated: * $p < 0.01$.
AD = Alzheimer's disease.

neurons within the DR and CSN of the macaque brainstem are serotonin-immunoreactive [R. Zweig and L. Walker, personal observations].

All NFTs within the designated areas were counted, both intracellular (irrespective of the size of the neuron or presence of neuromelanin) and extracellular. Thus, at any level, numbers of NFTs may reflect abnormalities of transmitter-specific neuronal populations with less certainty than counts of neurons.

Neuronal Loss and NFTs Within the LC in AD

The extent of neuronal loss within the LC in patients with AD in this study (counts 19–33% of control counts depending on level) is generally comparable with the findings of previous studies [4, 7–9, 11, 30]. The topography of this neuronal loss, i.e., greatest at mid level, is similar to that recently described by Marcynuik and associates [4] and is also consistent with a recent report describing relative sparing of posterior (caudal) LC neurons in AD [8]. As has been suggested [4], this pattern may indicate a preferential loss of neurons that project to brain regions, such as the neocortex, which are severely affected in AD [31].

The greater numbers of NFTs at mid level in comparison with the caudal level also suggests relatively less involvement caudally within this nucleus. This is particularly important because neuronal and NFT counts in individual patients were not correlated; that is, these measures appear to vary independently (see text following). The relatively large numbers of NFTs at the rostral level of LC (in comparison with numbers of neuromelanin-containing neurons) may reflect the large proportion of NFT-containing neurons at this level that do not contain neuromelanin. For example, some of the NFTs counted may have been associated with putative cholinergic neurons of the laterodorsal tegmental nucleus [32], which overlap in distribution with neuromelanin-containing neurons of the rostral LC.

Neuronal Loss and NFTs Within the Raphe Nuclei in AD

The moderate loss of neurons within the DR in those with AD in our study (counts 10–36% below control counts depending on level), lies intermediate between the 15% (not significant) loss of "large" neurons reported by Curcio and Kemper [1] and the 77% loss reported by Yamamoto and Hirano [5]. A more restricted population of larger neurons may have been counted in the Yamamoto and Hirano study; this would explain the similar control counts in the two studies despite a significantly larger nuclear boundary used by Yamamoto and Hirano. Qualitative rankings for numbers of "very large neurons" within small groups of cases with similar neuronal counts from our study (performed without knowledge of clinical status) revealed no differences between control subjects and patients with AD, suggesting that differences in our counts between those two groups would not have been greater if counts had been restricted to larger neurons. The rostral-to-caudal increase in neuronal loss in the DR might reflect topographical differences in projection patterns within this nucleus. Alternatively, level-to-level differences might reflect a different percentage of serotonergic neurons counted at each level.

In a previous study, a 36% loss of neurons (size not reported) and large numbers of NFTs in the CSN were noted in 4 patients with AD in comparison with 3 control subjects [10]. Our study does not confirm neuronal loss within this structure in AD, although these cells clearly show NFTs. Of note, this nucleus does project to the hippocampus and to other brain regions severely affected in AD [33].

Relationship between Pathology of Aminergic Systems and Depression

The finding that neuronal loss was significantly greater in depressed than in nondepressed patients with AD at the mid level of the LC and at the rostral level of the CSN represents the first demonstration of histological changes in the brain that relate to the presence of depression [21]. These results do not appear to reflect an interaction between depression and other factors measured such as MMS score, age, or duration of illness. Moreover, we noted a trend suggesting that depressed patients have a greater neuronal loss at all levels of the LC and the DR. This trend needs to be corroborated by additional studies, particularly because differences in age or MMS score or both may have contributed to this trend within the DR. In addition, other pathological measures, such as neuronal loss within the basal forebrain, need to be examined in depressed versus nondepressed patients with AD.

Our results are consistent with a variety of studies implicating noradrenergic and serotonergic systems in depression [24]. The results of neurochemical measurements of presynaptic and postsynaptic serotonin receptors and of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid or brain tissues have suggested alterations of serotonergic systems in depressed or suicidal patients [21, 34–39], as well as more severe serotonergic dysfunction in depressed (versus nondepressed) patients with Parkinson's disease [40–42]. Some depressed patients also have diminished renal excretion of the norepinephrine metabolite 3-methoxy-4-hydroxyphenylglycol [21, 43]. Antidepressant medications have a variety of effects on aminergic systems within the central nervous system [21, 44]. Moreover, serotonergic, and particularly noradrenergic, dysfunction has been demonstrated to occur in detailed studies of an animal model of depres-

sion [45, 46]. Altered catecholaminergic activity has also been demonstrated in an animal model of the depression that sometimes accompanies right hemispheric infarction [47, 48].

Other Clinical and Pathological Correlations in Patients with AD

Our study indicates that, in individual patients, the relative severity of neuronal loss or numbers of NFTs at one level of the LC or the DR reflects relative severity of involvement by these pathological processes at other levels of the same nucleus. However, correlations of numbers of neurons or counts of NFTs between nuclei in individual patients were, with several exceptions, much weaker. Study of a larger number of cases will be necessary to establish the significance of these correlations. The relative consistency of neuronal loss that we report within the LC has been previously demonstrated [6], as have correlations between neuronal loss in this nucleus with measures of forebrain abnormalities including neuronal loss within the nucleus basalis [9] and numbers of senile plaques and NFTs in the temporal cortex [13, 14]. Decreases in cortical levels of serotonin and 5-HIAA in cases of AD have also been found to correlate with numbers of NFTs in the temporal cortex [14].

No relationship was apparent between MMS score and neuronal loss in aminergic nuclei. Although this result may reflect insensitivity of the MMS scale in comparing cases with severe dementia, previous studies have also failed to demonstrate a correlation between measures of dysfunction of aminergic systems and extent of dementia [12, 14, 15]. The negative correlation between neuronal loss and age, particularly within the LC, is consistent with previous reports [11, 16], although the distribution of this relationship was continuous rather than bimodal as has been previously suggested [9]. There was no correlation between counts of NFTs and age. NFT counts correlated inversely with duration of illness, particularly within the DR. It is possible that patients whose illness was of longer duration had fewer NFTs than did those whose illness was of shorter duration because they were less severely demented, despite low MMS scores. A more attractive hypothesis, however, presumes that the similar (low) MMS scores among all but 3 of the patients reflect a similar severity of disease (as measured clinically). Then patients with a shorter duration would have had a more rapid progression of disease. Thus, numbers of NFTs within these nuclei might reflect rates of progression rather than severity of disease at the time of death.

In conclusion, the extent of neuronal loss within the LC, DR, or CSN may correspond better to the age of the patient and to the presence of depression than to the severity of dementia. In contrast, counts of NFTs may correspond best with the rate of disease progression.

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